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(54) Title: FIBROBLAST GROWTH FACTOR-10

(57) Abstract

Disclosed are human FGF-10 polypeptides and DNA (RNA) encoding such FGF-10 polypeptides. Also provided is a procedure for producing such polypeptide by recombinant techniques. Also disclosed are methods for utilizing such polypeptide for stimulating re-vascularization, for treating wounds and prevent neuronal damage. Antagonists against such polypeptides and their use as a therapeutic to prevent abnormal cellular proliferation, hyper-vascular diseases and epithelial lens cell proliferation are also disclosed. Diagnostic methods for detecting mutations in the FGF-10 coding sequence and alterations in the concentration of FGF-10 protein in a sample derived from a host are also disclosed.

FIBROBLAST GROWTH FACTOR-10

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are fibroblast growth factor-10/heparin binding growth factor-10, hereinafter referred to as "FGF-10". The invention also relates to inhibiting the action of such polypeptides.

Fibroblast growth factors are a family of proteins characteristic of binding to heparin and are, therefore, also called heparin binding growth factors (HBGF). Expression of different members of these proteins are found in various tissues and are under particular temporal and spatial control. These proteins are potent mitogens for a variety of cells of mesodermal, ectodermal, and endodermal origin, including fibroblasts, corneal and vascular endothelial cells, granulocytes, adrenal cortical cells, chondrocytes, myoblasts, vascular smooth muscle cells, lens epithelial cells, melanocytes, keratinocytes, oligodendrocytes, astrocytes, osteoblasts, and hematopoietic cells.

Each member has functions overlapping with others and also has its unique spectrum of functions. In addition to

glioma growth and progression independent of its role in tumor angiogenesis and that basic fibroblast growth factor release or secretion may be required for these actions (Morrison, R.S., et al., J. Neurosci. Res., 34:502-9 (1993)).

Fibroblast growth factors, such as basic FGF, have further been implicated in the growth of Kaposi's sarcoma cells *in vitro* (Huang, Y.Q., et al., J. Clin. Invest., 91:1191-7 (1993)). Also, the cDNA sequence encoding human basic fibroblast growth factor has been cloned downstream of a transcription promoter recognized by the bacteriophage T7 RNA polymerase. Basic fibroblast growth factors so obtained have been shown to have biological activity indistinguishable from human placental fibroblast growth factor in mitogenicity, synthesis of plasminogen activator and angiogenesis assays (Squires, C.H., et al., J. Biol. Chem., 263:16297-302 (1988)).

U.S. Patent No. 5,155,214 discloses substantially pure mammalian basic fibroblast growth factors and their production. The amino acid sequences of bovine and human basic fibroblast growth factor are disclosed, as well as the DNA sequence encoding the polypeptide of the bovine species.

The polypeptide of the present invention has been putatively identified as a member of the FGF family as a result of amino acid sequence homology with other members of the FGF family.

In accordance with one aspect of the present invention, there are provided novel mature polypeptides which are FGF-10 as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof. The polypeptides of the present invention are of human origin.

In accordance with one aspect of the present invention, there are provided isolated nucleic acid molecules encoding human FGF-10, including mRNAs, DNAs, cDNAs, genomic DNA, as

polypeptides, or polynucleotides encoding such polypeptides, for *in vitro* purposes related to scientific research, synthesis of DNA and manufacture of DNA vectors.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are meant only as illustrations of specific embodiments of the present invention and are not meant as limitations in any manner.

Figure 1 depicts the cDNA sequence and corresponding deduced amino acid sequence of FGF-10. The amino acid sequence shown represents the mature form of the protein. The standard one letter abbreviation for amino acids is used. Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.). Sequencing accuracy is predicted to be greater than 97% accurate.

Figure 2 illustrates the amino acid sequence homology between FGF-10 and the other FGF family members. Conserved amino acids are indicated in bold.

Figure 3 shows an SDS-PAGE gel after *in vitro* transcription/translation of FGF-10 protein.

In accordance with one aspect of the present invention, there are provided isolated nucleic acids molecules (polynucleotides) which encode for the mature polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID No. 2) or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75696 on March 4, 1994.

The polynucleotide of this invention was discovered initially in a cDNA library derived from 8 week old early stage human tissue and subsequently the full length cDNA was found in a library derived from the human Amygdala. It is structurally related to all members of the fibroblast growth

sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID No. 2) or the polypeptide encoded by the cDNA of the deposited clone. The variants of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in Figure 1 (SEQ ID No. 2) or the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of Figure 1 (SEQ ID No. 2) or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figure 1 (SEQ ID No. 1) or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a

same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1 (SEQ ID No. 1) or the deposited cDNA.

The deposit(s) referred to herein will be maintained under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the purposes of Patent Procedure. These deposits are provided merely as a convenience and are not an admission that a deposit is required under 35 U.S.C. § 112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with the description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to an FGF-10 polypeptide which has the deduced amino acid sequence of Figure 1 (SEQ ID No. 2) or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1 (SEQ ID No. 2) or that encoded by the deposited cDNA, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1 (SEQ ID No. 2) or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved

invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the FGF-10 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotide of the present invention may be employed for producing a polypeptide by recombinant techniques. Thus, for example, the polynucleotide sequence may be included in any one of a variety of expression vehicles, in particular vectors or plasmids for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector or plasmid may be used as long as they are replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease sites by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of

pNH16a, pNH18a, pNH46a (Stratagene); pTRC99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described construct. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, 1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived

more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a

conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions.

FGF-10 may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since it has the ability to be a mitogenic agent to various cell types, such as fibroblast cells and skeletal muscle cells.

FGF-10 may also be employed to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. FGF-10 has the ability to stimulate chondrocyte growth, therefore, it may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

FGF-10 may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

FGF-10 may also be employed for preventing hair loss, since FGF-10 activates hair-forming cells and promotes melanocyte growth. Along the same lines, FGF-10 stimulates growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

FGF-10 may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides, for *in vitro* purposes related to scientific research, synthesis of DNA, manufacture of DNA vectors and for the purpose of providing diagnostics and therapeutics for the treatment of human disease.

Fragments of the full length FGF-10 gene may be used as a hybridization probe for a cDNA library to isolate the full length FGF-10 gene and to isolate other genes which have a high sequence similarity thereto genes or which have similar

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

This invention provides a method of screening compounds to identify those which modulate the action of FGF-10. An example of such an assay comprises combining a mammalian fibroblast cell, FGF-10, the compound to be screened and ^{3}[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation of Keratinocytes by determining the uptake of ^{3}[H] thymidine in each case.

To screen for antagonists, the same assay may be prepared and the ability of the compound to prevent fibroblast proliferation is measured and a determination of antagonist ability is made. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of ^{3}[H] thymidine.

In another method, a mammalian cell or membrane preparation expressing the FGF-10 receptor would be incubated with labeled FGF-10 in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of FGF-10

Potential FGF-10 antagonists include small molecules which bind to and occupy the binding site of the FGF-10 receptor thereby making the receptor inaccessible to FGF-10 such that normal biological activity is prevented. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules.

FGF-10 antagonists may be employed to inhibit cell growth and proliferation effects of FGF-10 on neoplastic cells and tissues and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, the growth of tumors.

FGF-10 antagonists may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery.

The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The polypeptides, agonists and antagonists of the present invention may be employed in combination with a suitable pharmaceutical carrier to comprise a pharmaceutical composition. Such compositions comprise a therapeutically effective amount of the polypeptide, agonist or antagonist and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In

expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such methods should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retroviral particle, for example, an adenovirus, which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

This invention is also related to the use of the FGF-10 gene as part of a diagnostic assay for detecting diseases or susceptibility to diseases related to the presence of mutations in the FGF-10 nucleic acid sequences.

Individuals carrying mutations in the FGF-10 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki *et al.*, Nature, 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding FGF-10 can be used to identify and analyze FGF-10 mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled FGF-10 RNA or alternatively, radiolabeled FGF-10 antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA

host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein like bovine serum albumen. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any FGF-10 proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to FGF-10. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of FGF-10 protein present in a given volume of patient sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to FGF-10 are attached to a solid support and labeled FGF-10 and a sample derived from the host are passed over the solid support and the amount of label detected, for example by liquid scintillation chromatography, can be correlated to a quantity of FGF-10 in the sample.

A "sandwich" assay is similar to an ELISA assay. In a "sandwich" assay FGF-10 is passed over a solid support and binds to antibody attached to a solid support. A second antibody is then bound to the FGF-10. A third antibody which is labeled and specific to the second antibody is then passed over the solid support and binds to the second antibody and an amount can then be quantified.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data

good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

Example 2

Expression of FGF-10 by *in vitro* transcription and translation.

The FGF-10 cDNA, ATCC # 75696, was transcribed and translated *in vitro* to determine the size of the translatable polypeptide encoded by the full length and partial FGF-10 cDNA. The full length and partial cDNA inserts of FGF-10 in the pBluescript SK vector were amplified by PCR with three pairs of primers, 1) M13-reverse and forward primers; 2) M13-reverse primer and FGF primer P20; 3) M13-reverse primer and FGF primer P22. The sequence of these primers as follows.

M13-2 reverse primer:

5'-ATGCTTCCGGCTCGTATG-3' (SEQ ID No. 3)

This sequence is located upstream of the 5' end of the FGF-10 cDNA insert in the pBluescript vector and is in an anti-sense orientation with respect to the cDNA. A T3 promoter sequence is located between this primer and the FGF-10 cDNA.

M13-2 forward primer:

5'-GGGTTTTCCCAGTCACGAC-3' (SEQ ID No. 4)

This sequence is located downstream of the 3' end of the FGF-10 cDNA insert in the pBluescript vector and is in an anti-sense orientation with respect to the cDNA insert.

FGF primer P20:

5'-GTGAGATCTGAGGGAAGAAGGGGA-3' (SEQ ID No. 5)

The 15 bp sequence of this primer on the 3' prime is anti-sense to the FGF-10 cDNA sequence bp 780-766, which is 12 bp downstream from the stop codon.

FGF primer P22:

5'-CCACCGATAATCCTCCTT-3' (SEQ ID No. 6)

This sequence is located within the FGF-10 cDNA in an anti-sense orientation and is about 213 bp downstream from the stop codon.

PCR reaction with all three pairs of primers produce amplified products with T3 promoter sequence in front of the

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HU, ET AL.

(ii) TITLE OF INVENTION: Fibroblast Growth Factor-10

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

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(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: Concurrently
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: 08/207,412
(B) FILING DATE: 8 MAR 1994

ATAAATATTA AACTAAACTG TATTGTTATT AGTAGAAGGC TAATTGTAAT GAAGACATTA 1080
ATAAAGGTGA AATAAACTTA AAAAAAAAAA AAAAAAAAAA A 1121

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 181 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Ser Lys Glu Pro Gln Leu Lys Gly Ile Val Thr Arg Leu
5 10 15
Phe Ser Gln Gln Gly Tyr Phe Leu Gln Met His Pro Asp Gly Thr
20 25 30
Ile Asp Gly Thr Lys Asp Glu Asn Ser Asp Tyr Thr Leu Phe Asn
35 40 45
Leu Ile Pro Val Gly Leu Arg Val Val Ala Ile Gln Gly Val Lys
50 55 60
Ala Ser Leu Tyr Val Ala Met Asn Gly Glu Gly Tyr Leu Tyr Ser
65 70 75
Ser Asp Val Phe Thr Pro Glu Cys Lys Phe Lys Glu Ser Val Phe
80 85 90
Glu Asn Tyr Tyr Val Ile Tyr Ser Ser Thr Leu Tyr Arg Gln Gln
95 100 105
Glu Ser Gly Arg Ala Trp Phe Leu Gly Leu Asn Lys Glu Gly Gln
110 115 120
Ile Met Lys Gly Asn Arg Val Lys Lys Thr Lys Pro Ser Ser His
125 130 135
Phe Val Pro Lys Pro Ile Glu Val Cys Met Tyr Arg Glu Pro Ser
140 145 150
Leu His Glu Ile Gly Glu Lys Gln Gly Arg Ser Arg Lys Ser Ser
155 160 165

(A) LENGTH: 24 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGAGATCTG AGGGAAGAAG GGGA 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCACCGATAA TCCTCCTT 18

9. A vector containing the DNA of Claim 2.

10. A host cell genetically engineered with the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 9.

13. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having FGF-10 activity.

14. A polypeptide selected from the group consisting of: (i) a polypeptide having the deduced amino acid sequence of SEQ ID No. 2 and fragments, analogs and derivatives thereof and (ii) a polypeptide encoded by the cDNA of ATCC Deposit No. 75696 and fragments, analogs and derivatives of said polypeptide.

15. The polypeptide of Claim 14 wherein the polypeptide is FGF-10 having the deduced amino acid sequence of SEQ ID No. 2.

16. An antibody against the polypeptide of claim 14.

17. A compound effective as an agonist to the polypeptide of claim 14.

18. A compound effective as an antagonist against the polypeptide of claim 14.

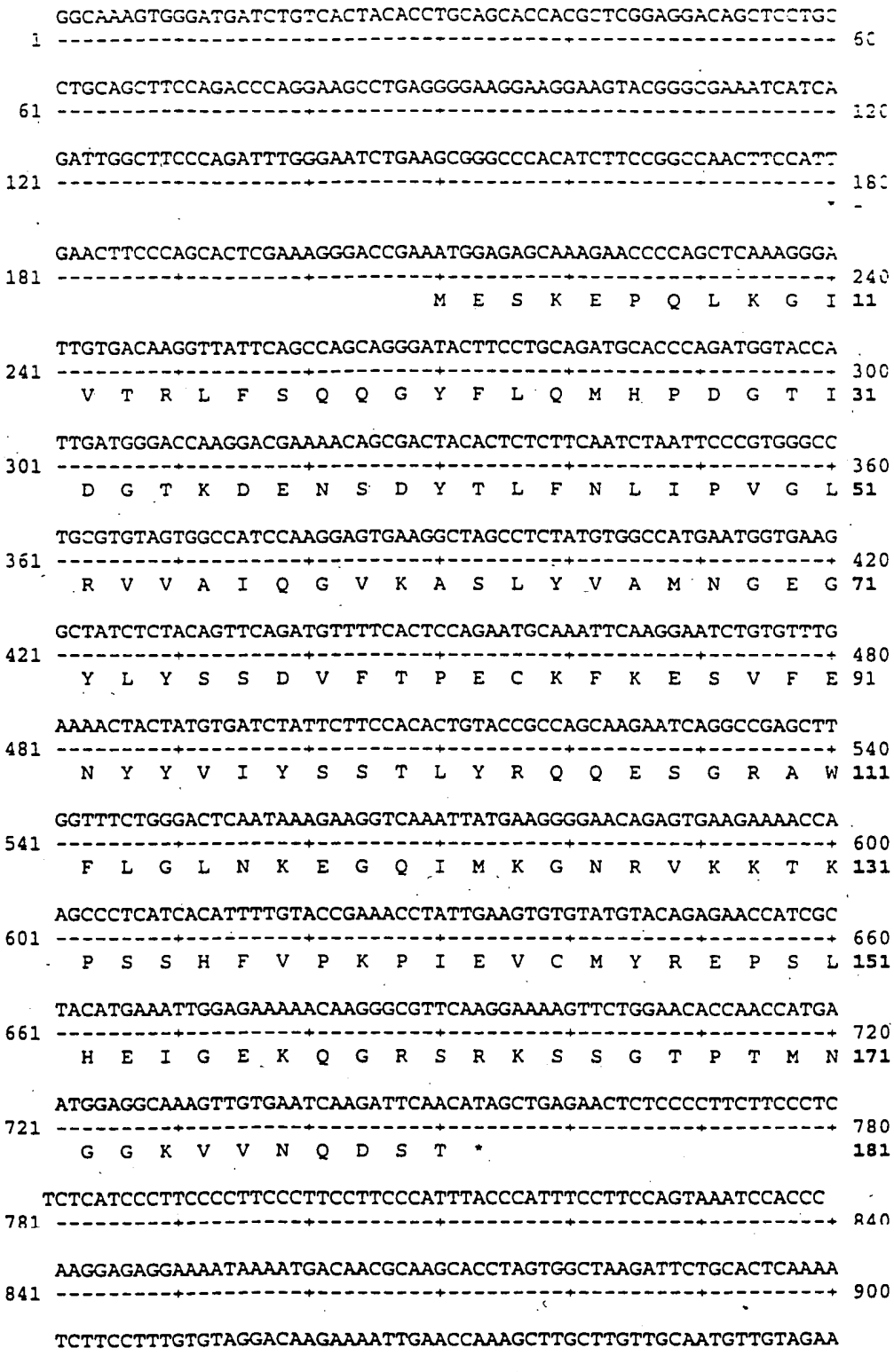

GGCAAAGTGGGATGATCTGTCACTACACCTGCAGCACCACGCTCGGAGGACAGCTCCTGC
1 ---------- 60
CTGCAGCTTCCAGACCCAGGAAGCCTGAGGGGAAGGAAGGAAGTACGGGCGAAATCATCA
61 ---------- 120
GATTGGCTTCCCAGATTTGGGAATCTGAAGCGGGCCCACATCTTCCGGCCAACTTCCATT
121 ---------- 180
GAACTTCCCAGCACTCGAAAGGGACCGAAATGGAGAGCAAAGAACCCCAGCTCAAAGGGA
181 ---------- 240
M E S K E P Q L K G I 11
TTGTGACAAGGTTATTCAGCCAGCAGGGATACTTCCTGCAGATGCACCCAGATGGTACCA
241 ---------- 300
V T R L F S Q Q G Y F L Q M H P D G T I 31
TTGATGGGACCAAGGACGAAAACAGCGACTACACTCTCTTCAATCTAATTCCCGTGGGCC
301 ---------- 360
D G T K D E N S D Y T L F N L I P V G L 51
TGCGTGTAGTGGCCATCCAAGGAGTGAAGGCTAGCCTCTATGTGGCCATGAATGGTGAAG
361 ---------- 420
R V V A I Q G V K A S L Y V A M N G E G 71
GCTATCTCTACAGTTCAGATGTTTTCACTCCAGAATGCAAATTCAAGGAATCTGTGTTTG
421 ---------- 480
Y L Y S S D V F T P E C K F K E S V F E 91
AAAACTACTATGTGATCTATTCTTCCACACTGTACCGCCAGCAAGAATCAGGCCGAGCTT
481 ---------- 540
N Y Y V I Y S S T L Y R Q Q E S G R A W 111
GGTTTCTGGGACTCAATAAAGAAGGTCAAATTATGAAGGGGAACAGAGTGAAGAAAACCA
541 ---------- 600
F L G L N K E G Q I M K G N R V K K T K 131
AGCCCTCATCACATTTTGTACCGAAACCTATTGAAGTGTGTATGTACAGAGAACCATCGC
601 ---------- 660
P S S H F V P K P I E V C M Y R E P S L 151
TACATGAAATTGGAGAAAAACAAGGGCGTTCAAGGAAAAGTTCTGGAACACCAACCATGA
661 ---------- 720
H E I G E K Q G R S R K S S G T P T M N 171
ATGGAGGCAAAGTTGTGAATCAAGATTCAACATAGCTGAGAACTCTCCCCTTCTTCCCTC
721 ---------- 780
G G K V V N Q D S T * 181
TCTCATCCCTTCCCCTTCCCTTCCTTCCCATTTACCCATTTCCTTCCAGTAAATCCACCC
781 ---------- 840
AAGGAGAGGAAAATAAAATGACAACGCAAGCACCTAGTGGCTAAGATTCTGCACTCAAAA
841 ---------- 900
TCTTCCTTTGTGTAGGACAAGAAAATTGAACCAAAGCTTGCTTGTTGCAATGTTGTAGAA

FIGURE 1 1/2

```
 901 ------------------------------------------------------------ 960

     AATTCACGTTCACAAAGATTATCACACTTAAAAGCAAAGGAAAAAATAAATCAGAACTCC
 961 ------------------------------------------------------------ 1020

     ATAAATATTAAACTAAACTGTATTGTTATTAGTAGAAGGCTAATTGTAATGAAGACATTA
1021 ---------+----------+----------+----------+----------+------ 1080

     ATAAAGGTGAAATAAACTTAAAAAAAAAAAAAAAAAAAAA
1081 ---------+----------+----------+---------- 1121
```

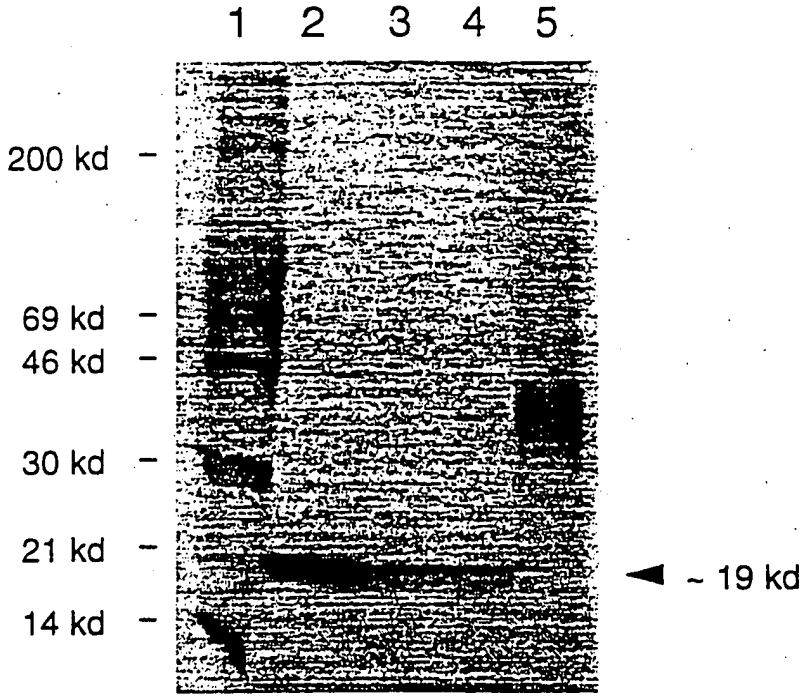
FIGURE 1 2/2

```
                                                                      50
          1
Fgf-1     .......... .......... .......... .......... ..........
Fgf-2     .......... .......... ..LGDRGRGR ALPGGRLGGR GRGRAPERVG
Fgf-4     .......... MS.GPGTAAV ALLPAVLLAL LA........ .PWAGRGGAA
Fgf-6     MALGQKLFIT MSRGAGRLQG TLWALVFLGI LV........ .GMVVPSPAG
Fgf-5     .......... .......MSL SFLLLLFFSH LILSAWAHGE KRLAPKGQPG
Fgf-9     .......... .......... .......... .......... ..MAPLGEVG
Fgf10     .......... .......... .......... .......... ..........
Fgf-3     .......... .......... .......... .......... ..........
Fgf-7     .......... .......... .......... .........M HKWILTWILP

                                                                     100
          51
Fgf-1     .......... .......... .......MAE GEITTFTALT EKFN...LPP
Fgf-2     GRGRGRGTAA PRAAPAARGS RPGPAGTMAA GSITTLPALP EDGGSGAFPP
Fgf-4     APTAPNGTLE AELERRWESL VALSLARLPV AA..QPKEAA VQSGAGDYLL
Fgf-6     TR.ANNTLLD S...RGWGTL LSRSRAGLAG EI......AG VNWESG.YLV
Fgf-5     PAATDRNPIG SSSRQSSSSA MSSSSASSSP AASLGSQGSG LEQSSFQWSP
Fgf-9     NYFGVQDAVP FGNVPVLPVD SPVLLSDHLG QSEAGGLPRG PAVTDLDHLK
Fgf10     .......... .......... .......... .......... .MESKEPQLK
Fgf-3     .......... MGLIWLLLLS LLEPGWPAAG PGARLRRDAG GRGGVYEHLG
Fgf-7     TLLYRSCFHI ICLVGTISLA CNDMTPEQMA TNVNCSSPER HTRSYDYMEG

                                                                     150
          101
Fgf-1     GNYKKPKLLY CSNGGHFLRI LPDGTVDGTR DRSDQHIQLQ LSAESVGEVY
Fgf-2     GHFKDPKRLY CKNGGFFLRI HPDGRVDGVR EKSDPHIKLQ LQAEERGVVS
Fgf-4     GIKRLRRLYC NVGIGFHLQA LPDGRIGGAH ADT.RDSLLE LSPVERGVVS
Fgf-6     GIKRQRRLYC NVGIGFHLQV LPDGRISGTH EEN.PYSLLE ISTVERGVVS
Fgf-5     SGRRTGSLYC RVGIGFHLQI YPDGKVNGSH EAN.MLSVLE IFAVSQGIVG
Fgf-9     GILRRRQLYC RT..GFHLEI FPNGTIQGTR KDHSRFGILE FISIAVGLVS
Fgf10     GIVTR..LFS QQ..GYFLQM HPDGTIDGTK DENSDYTLFN LIPVGLRVVA
Fgf-3     GAPRRRKLYC AT..KYHLQL HPSGRVNGSL .ENSAYSILE ITAVEVGIVA
Fgf-7     GDIRVRRLFC RT..QWYLRI DKRGKVKGTQ EMKNNYNIME IRTVAVGIVA

                                                                     200
          151
Fgf-1     IKSTETGQYL AMDTDGLLYG SQTPNEECLF LERLEENHYN TYISKKH...
Fgf-2     IKGVCANRYL AMKEDGRLLA SKCVTDECFF FERLESNNYN TYRSRKY...
Fgf-4     IFGVASRFFV AMSSKGKLYG SPFFTDECTF KEILLPNNYN AYESYKY...
Fgf-6     LFGVRSALFV AMNSKGRLYA TPSFQEECKF RETLLPNNYN AYESDLY...
Fgf-5     IRGVFSNKFL AMSKKGKLHA SAKFTDDCKF RERFQENSYN TYASAIH...
Fgf-9     IRGVDSGLYL GMNEKGELYG SEKLTQECVF REQFEENWYN TYSSNLY...
Fgf10     IQGVKASLYV AMNGEGYLYS SDVFTPECKF KESVFENYYV IYSSTLY...
Fgf-3     IRGLFSGRYL AMNKRGRLYA SEHYSAECEF VERIHELGYN TYASRLYRTV
Fgf-7     IKGVESEFYL AMNKEGKLYA KKECNEDCNF KELILENHYN TYASAKW...

                                                                     250
          201
Fgf-1     .......... .AEKNWFVGL KKNGSCKRG. .PRTHYGQKA ILFLPLPVSS
Fgf-2     .......... .T..SWYVAL KRTGQYKLG. .SKTGPGQKA ILFLPMSAKS
Fgf-4     .......PGM ......FIAL SKNGKTKKG. .NRVSPTMKV THFLPRL...
Fgf-6     .......QGT ......YIAL SKYGRVKRG. .SKVSPIMTV THFLPRI...
Fgf-5     .......RTE KTGREWYVAL NKRGKAKRGC SPRVKPQHIS THFLPRFKQS
Fgf-9     .......KHV DTGRRYYVAL NKDGTPREG. .TRTKRHQKF THFLPRPVD.
Fgf10     .......RQQ ESGRAWFLGL NKEGQIMKG. .NRVKKTKPS SHFVPKPIE.
Fgf-3     SSTPGARRQP SAERLWYVSV NGKGRPRRG. .FKTRRTQKS SLFLPRVLDH
Fgf-7     .........T HNGGEMFVAL NQKGIPVRG. .KKTKKEQKT AHFLPMAIT.
```

FIGURE 2 1/2

```
        251                                                        300
Fgf-1   D.......... .......... .......... .......... ..........
Fgf-2   .......... .......... .......... .......... ..........
Fgf-4   .......... .......... .......... .......... ..........
Fgf-6   .......... .......... .......... .......... ..........
Fgf-5   EQPELSFTVT VPEKKNPPSP IKSKIPLSAP RKNTNSVKYR LKFRFG....
Fgf-9   .......... ..PDKVPELY KDILSQS... .......... ..........
Fgf10   .......... ..VCMYREPS LHEIGEKQGR SRKSSGTPTM NGGKVVNQDS
Fgf-3   RDHEMVRQLQ SGLPRPPGKG VQPRRRRQKQ SPDNLEPSHV QASRLGSQLE
Fgf-7   .......... .......... .......... .......... ..........

        301
Fgf-1   ....
Fgf-2   ....
Fgf-4   ....
Fgf-6   ....
Fgf-5   ....
Fgf-9   ....
Fgf10   T*..
Fgf-3   ASAH
Fgf-7   ....
```

FIGURE 2 2/2



Lane 1: 14-C & rainbow M.W. marker
Lane 2: FGF10 (M13-reverse & forward primers)
Lane 3: FGF10 (M13-reverse & FGF-P20 primers)
Lane 4: FGF10 (M13-reverse & FGF-P22 primers)
Lane 5: FGF control

FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02950

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/00; A61K 38/18; C07K 14/50

US CL :530/399; 536/23.5; 435/69.4, 320.1, 252.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399; 536/23.5; 435/69.4, 320.1, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, dialog, Genbank

search terms: FGF-10

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,868,113 (JAYE ET AL.) 19 September 1989, figure 8.	13
X	Science, Volume 233, issued 1 August 1986, M. Jaye et al., "Human Endothelial Cell Growth Factor: Cloning, Nucleotide Sequence, and Chromosome Localization," pages 541-545, especially figure 2.	13
X	Annals New York Academy of Sciences, Volume 638, issued 1991, S.A. Aaronson et al., "Keratinocyte Growth Factor," pages 62-77, especially figure 2.	13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 MAY 1995

Date of mailing of the international search report

30 MAY 1995

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Form PCT/ISA/210 (second sheet)(July 1992)*

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.
Group I, claims 1-13, drawn to a polynucleotide encoding FGF-10, a vector, host cells, and a method of producing the protein.
Group II, claims 14-15, 19, and 21, to drawn to an FGF-10 polypeptide, a method of treatment, and a pharmaceutical composition, and a method of use.
Group III, claims 16-18, 20, drawn to an antibody and a method of use.
Group IV, claim 22, drawn to a method of identifying compounds active as antagonists or antagonists.
Group V, claims 23-24, drawn to a method of diagnosing a disease.
The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Inventions I and II are related as a DNA molecule which encodes a protein molecule. The purpose of the DNA molecule is to produce the protein in recombinantly produced host cell. Although the DNA molecule and the protein are related since the DNA encodes for the specific claimed protein, they do not share a special technical feature, as the protein product can be made by another and materially different process, such as by synthetic peptide synthesis.
The invention of group III is related to the inventions of groups II and I as an antibody raised to a protein (group II) and the DNA which encodes the protein (group I). Although immunologically related, the inventions comprise distinct products as evidenced by their primary, secondary, and tertiary structure, which do not share a special technical feature. The invention of group IV is related to the inventions of group III or group I as a method of using the products, and the invention of group V is related to the inventions of group II or III as a method of using the product. The inventions of groups IV and V represent distinct methods having different purposes and do not share a special technical feature. However, note that PCT Rule 13 does not provide for multiple products or methods of use within a single application.